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THE DOUBLE INTRADERMIC TEST IN CATTLE.

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Our interest in the double intradermic tuberculin cattle test arose from a suggestion which had reached us that a given tuberculin, which on the official guinea pig test had been proved equal to the (Koch's) Frankfort standard, might fail to give "positive" reactions in tuberculous cattle when used in the double intradermic test, while another sample similarly standardised gave definite reactions. (We may here anticipate our findings by stating that we found no evidence for the truth of the suggestion.) We soon found that we had to investigate many points before we could get a satisfactory answer to our question, so that our enquiry, instead of being merely an investigation of tuberculin, had to become an investigation of the consistency of conclusions drawn from the test.

Owing to the broadminded attitude and friendly interest of several owners of dairy herds within reasonable distance of our laboratory, we were able to carry out intradermic work on a mixed dairy herd including milch cows, pregnant cows, heifers, calves and bulls. Our investigations were limited because we were not able to carry out many subcutaneous tests, nor were we able to kill cattle. The restrictions under which we worked must be borne in mind when reading this report. We carried out in all between 400 and 500 tests on 150 animals, mostly cows and heifers.

We have reason to believe that the tuberculin we used in most of this work has proved itself a satisfactory tuberculin for the detection of tuberculosis in cattle.

We are in general agreement with the Tuberculin Committee that the double intradermic test is a convenient one under certain circumstances, and has "come to stay." It is, therefore, of considerable importance to the veterinary research worker and to the veterinary surgeon, acting for the dairy farmer, to define as closely as possible the limitations of the test and its degree of accuracy. We set out with the idea that we had simply to find some dozen "positive reactors" to the test and acquire these as a laboratory test herd of tuberculous animals for repeated experiments in the comparison of various experimental tuberculins in which we were interested. Our early tests, however, indicated that a cow giving a "strong positive" reaction might, a fortnight later, fail to show the same degree of reaction to the same tuberculin. We had, therefore, to extend our work, for we found that we could not retest in the sam animal a point that arose and be certain that the answer in the retes would be the same as it would have been in an original test.

Throughout this report, for the sake of clearness, a double intra dermic test is termed D.I.T. It consist of a first injection (F.I. and a repeat injection (R.I.) 48 hours later. If, at a subsequent dat another double intradermic test is carried out in the same anima we call it a second double intradermic test or second D.I.T. B first difference (F.D.) we mean the difference between the origina thickness of the skin and the thickness after the first injection, *i.e.*, th increase by the reaction following the injection: second differenc (S.D.) represents the difference between the original thickness the skin and that observed after the repeat injection.

I.—The Error of the Test.

In our early tests, carried out over two years ago, we formed th impression that size of swelling produced by the D.I.T. might b taken as an indication of the reaction, though naturally its charact and the amount of tenderness and heat were also noted. It soo became evident, however, that the increase in skin thickness alon would not always enable one to make a diagnosis, but that th character of the swelling, tenderness, etc., must all be taken in account.

From a study of many tests we have laid down for ourselves th following rules in reading the results of reactions: (*a*) To be "positive" reactor a cow must show S.D. of at least 8 mm.; the must be tenderness, recorded as flinching, not when the skin adjace to the injected area is first gently pinched, but definitely when th injected area is gently manipulated; there must be a palpab thickening with indefinite edges. The question of heat in the inject area cannot always be clearly defined.

In passing we would note that we have practically never fou an animal which on character of swelling we wished to place in t "positive" class, unless there was also a large F.D. or S.D.; furth our impression is that a large swelling, *e.g.*, F.D. or S.D.=20 m would in practically all cases indicate a "positive" reaction, thou character of swelling had not been recorded. These impressio have been formed by the careful checking of the results of many tes (*b*) To be "negative" to the D.I.T. a cow must not show an S.D. more than 10 mm.; there must be no tenderness when tested already described; the swelling must be either practically non-existe or hard and sharply circumscribed. No heat should be detect

c) If the reading of the test of any animal failed to satisfy us that a definitely "positive" or "negative" result had been got, the animal was classified as "doubtful" and must be submitted to a econd D.I.T. at some future date. We do not suggest that these hould be accepted as general rules; it proved convenient in practice hat we should, for our own guidance, lay down as definite rules as possible. Different operators would place the exact limits at points orresponding to their personal experience.

In our opinion the actual operation of injecting is most important. The same injector will not always introduce the tuberculin into the same layer of skin in the same cow and as the original skin thickness n cattle varies considerably, some variation in the actual location of the injected tuberculin must exist.

On many occasions the same tuberculin has been injected by the same injector into three adjacent sites on the neck of a cow. Mostly the three readings obtained would be equal within the limits of experimental error, but occasionally a discrepancy might occur which might cause an observer to place the reactions in different classes. Table I indicates that in one series of 18 cows so tested, eight cows showed definite positive reactions to all three tests, four were definitely negative, one gave three doubtful reactions, while in five cows the readings failed to classify the cows as definite "positive" reactors to all three tests.

The reading of reactions has been carefully studied. To check the accuracy of readings we adopted the following procedure: The reactions of the D.I.T. were read by observer A and recorded in a notebook by B. After completing the readings of the whole herd under test, A went over every cow again and gave a second reading without knowing what his first reading was. Repeat readings were now made by B, while A recorded. In a series of 71 readings the agreements were, on the whole, satisfactory. The greatest difference in size of the S.D. made by A was 3 mm. and B 4 mm. The character of the swelling and tenderness agreed in most cases and the final verdict, "positive," "negative" or "indefinite" agreed in 62 of 71 readings. The greatest discrepancies occurred in the "negative" and "indefinite" groups, for in the "undoubtedly negative" or "strongly positive" cows there is rarely room for any difference of opinion between different observers making a reading on the same cow. There was nothing to choose between the accuracy of A and B. Table II shows the readings on two cows injected with the same tuberculin in three sites; they are chosen because of the unusually large variations in the readings.

TABLE I.

Final Diagnosis on Three Injections.	Number of Cows.
All 3+	8
All 3—	4
2 ? 1+	3
2+ 1 ?	2
All 3 ?	1

+ =Positive Reaction. — =Negative Reaction.
? =Doubtful Reaction.

TABLE II.

	Observer.	S.D.	Character of Swelling.	Tenderness.	Diagnosis
Cow 9 ..	A	14	D	—	?+
		13	D—	—	?
		11	D—	—	?
	B	13	D	—	?—
		10	D	—	?—
		9	D—	—	?—
Cow 10 ..	A	12	D—	+	+
		10	D	+	+
		8	D—	+	?
	B	11	D	+	+
		10	D	+	++
		5	D	+	?+

Final conclusion Cow 9, " probably negative "; 10 " positive."
Third injection in Cow 10 presumably too deep.
S.D.=increase in skin thickness between original and after secon injection (R.I.).
D.=Diffuse swelling.
D—=Indefinite, not clearly diffuse.

CONCLUSIONS.—The error of the D.I.T. may occasionally be considerable in that the same injector may obtain varying results wit the same tuberculin in the same cow. The reading of the results also subject to variation, especially when " doubtful " reactions a got. With reasonable care and experience these errors can, in practic be minimised.

II.—Types of Reaction.

Our experiments indicate that three types of reaction exist : positive," " negative " and " indefinite."

There is no hard and fast rule as to what constitutes a positive a negative reaction, but from the rules we have laid down for our- lves (see above), we have generally had no difficulty in deciding which of these two groups a reaction must be placed. The F.I. varying occasions would appear to be sufficient to arrive at a finite conclusion as to positive reaction ; our present impression that 5 to 15 per cent. of our cows which were read as positive on e R.I. would have been recorded as " doubtful " or " negative " ter the F.I.

There is, however, a group of intermediate cows, which give ecidedly indefinite reactions after the R.I. The percentage varies om herd to herd, and our figures are not large enough to enable us give a definite percentage. Our experiments have not been in ogress a sufficient time to enable us to decide whether an " indefinite " actor will ultimately become a " positive " reactor, but we found at some of our cows gave indefinite reactions when again tested a ear later. We retested 12 such " indefinite " reactors by D.I.T. vo to four weeks after the first indefinite reaction was obtained. n every occasion the second reaction was also " indefinite."

We have also records of the testing by the subcutaneous tuberculin ethod of such " indefinite " reactors. The subcutaneous tests were rried out at least one month after the D.I.T., and of eight cows tested three reacted to the subcutaneous tests, one gave a " doubtful" action and four gave negative results. These cows are still under servation and will be retested by both methods in due course.

Conclusions.—An intermediate group of reactions has been met ith to which the terms " doubtful " or " indefinite " may be applied.

III.—Specificity of the Reaction.

An increase in swelling and tenderness after the R.I. of tuberculin specific, *i.e.*, it is not produced if the R.I. consists of plain broth concentrated glycerine veal broth containing no " tuberculin."

This point has been tested on several occasions. In one experiment 2 cows, six " positive " and six " negative " reactors, were injected three sites with an F.I. of the same tuberculin. For the R.I. we jected tuberculin into one area and concentrated glycerine veal roth into the second, while the third we left untouched. The R.I. glycerine veal broth produced little change in the character of he reaction caused by the F.I. tuberculin. Where no R.I. was given he character of the reaction was more or less unchanged when readings were made at the end of 72 hours, while the reactions caused by the R.I. of the tuberculin were definitely increased in all cases. Chart I shows results of several such tests.

Conclusions.—Our results are, therefore, in complete agreement with those of the Tuberculin Committee: the reaction produced by the repeat injection (R.I.) is specific.

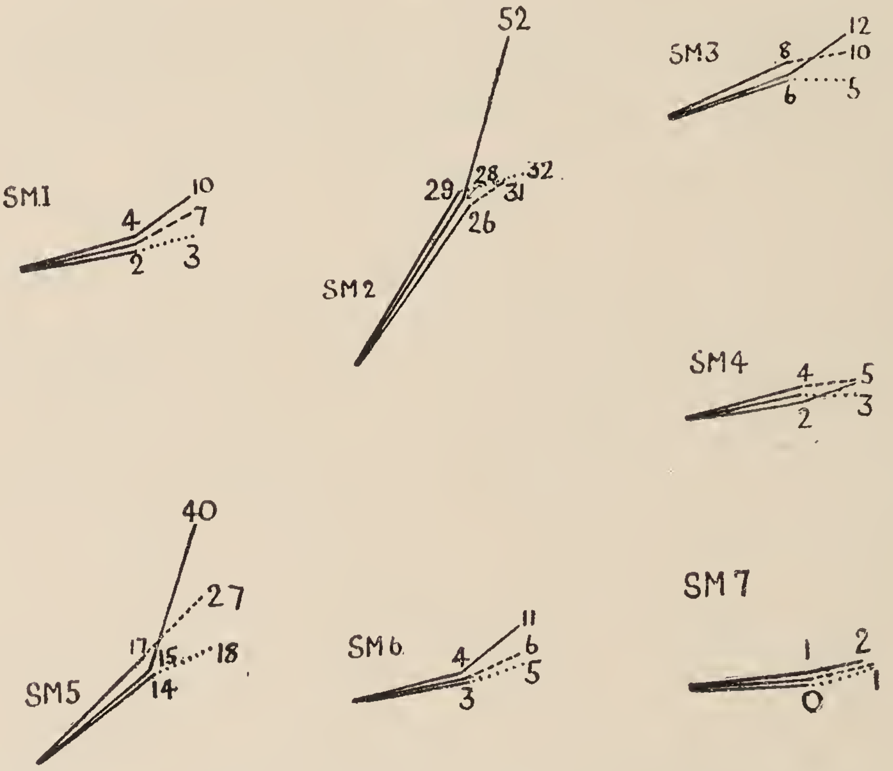

CHART I. Specificity of reaction following the R.I.

Continuous line = Tuberculin F.I. and R.I.
Broken line = ,, ,, only.
Dotted line = Tuberculin F.I. and glycerine veal broth R.I.
Figures represent increase in thickness of skin in millimetres.

IV.—COMPARING TUBERCULINS WHICH ARE EQUAL IN POTENCY B THE OFFICIAL FRANKFORT METHOD, BY D.I.T. IN CATTLE.

The fundamental problem we set out to solve was whether th potency of two tuberculins measured by the D.I.T. corresponded t that obtained by the official Frankfort method.

We have carried out many tests on this point, and our definit conclusion is that if two tuberculins tested by the official Frankfor method and found to be equal in potency to standard are compare by the D.I.T., they will also by the latter test prove of equal potency Nineteen pairs of tuberculin have been so tested and general agreemen has been obtained. Our experience convinces us that a large numbe of "reacting" cows must be used to determine the point. A erroneous conclusion may be reached if a small number of cows b used, especially if degrees of reaction are considered. One examp will serve to indicate how such erroneous deductions may be mad A herd of mixed cattle was injected, each receiving a dose of tw different tuberculins, proved equal in potency to standard on man casions over a long period by the Frankfort official test. The jections were made on adjacent parts of the same side of the neck each animal. The injector was not aware which tuberculin he was jecting into any special site. The R.I. was made after 48 hours,

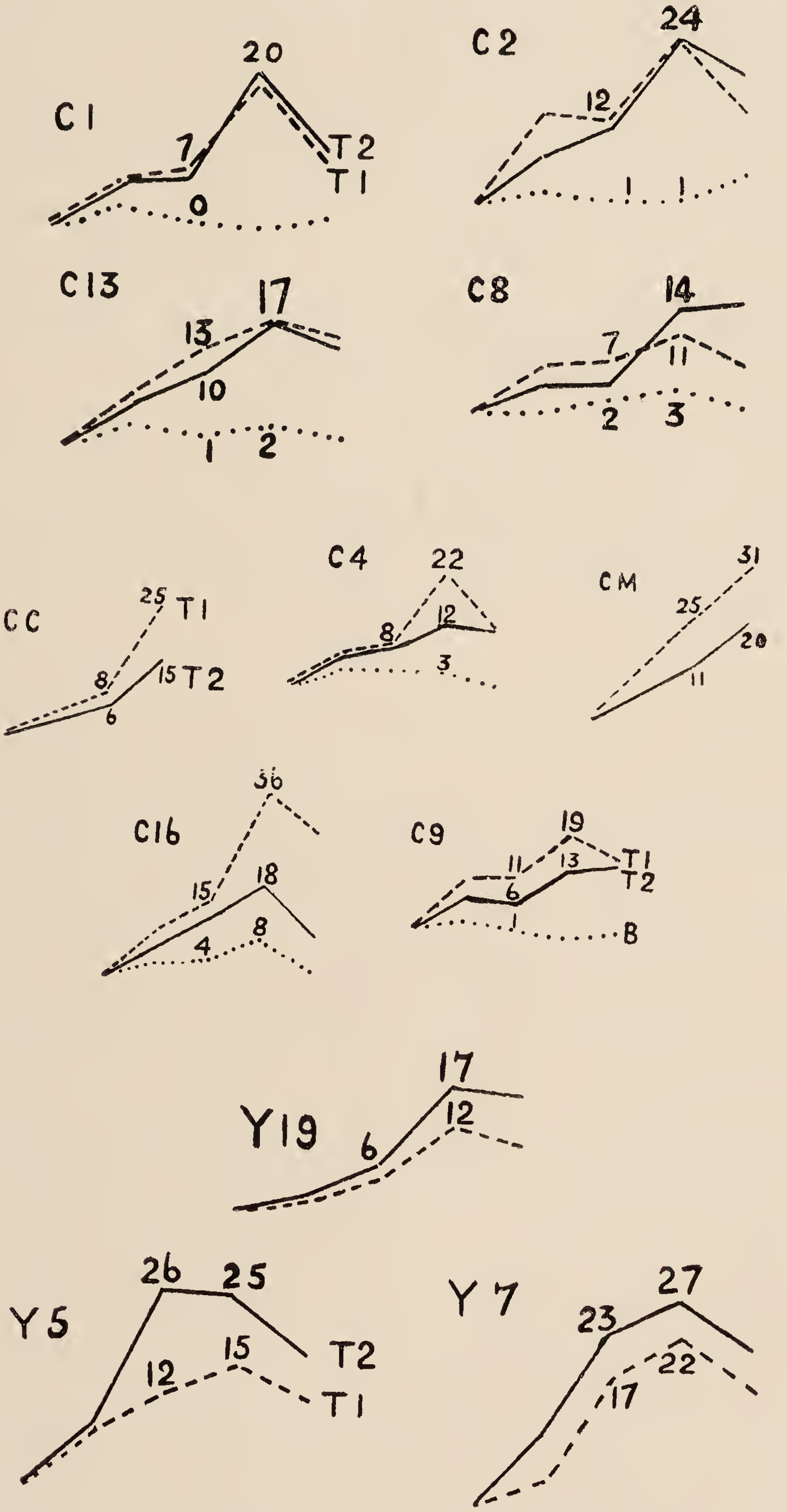

CHART 2. Comparison of Tuberculin by I.D. Test in Cattle.

Broken Line = Tuberculin T.1 for F.I. and R.I.
Continuous Line = ,, T.2 ,, ,, ,, ,,
Dotted line = Glycerine veal broth for F.I. and R.I.
Figures represent increase in thickness of skin in millimetres.

the same tuberculin being used in the same site. Twenty-two cows gave positive reactions, which are diagrammatically shown in Chart II. In 14 cases, of which C1, C2, C8 and C13 are examples, there is little difference in the degree of reaction as judged by F.D. and S.D., *i.e.*, T1 is equal to T2, while in five cows, CC, C4, CM, C9 and C16, one tuberculin gave a decidedly better reaction than the other (*i.e.*, T1 greater than T2) and in three cows, Y5, Y7 and Y19, the opposite (*i.e.*, T1 less than T2) held good.

Summary and Conclusions.—Tuberculins proved equal in potency by the official Frankfort method, and by the intradermic test in guinea pigs are also equal by D.I.T. in cattle. To make a satisfactory comparison in cattle a number of cows must be used.

For titration of tuberculins the only satisfactory method at presen available is the intradermic test in guinea pigs followed by the subcutaneous test.

We are in agreement with the view arising from the article b Buxton (*Vet. Record*, 1925, July, 18th) that in practical work it wil increase the confidence of the veterinary surgeon if the tuberculi he is using for the test has also been shown to produce satisfactor reactions in cattle.

V.—Potency of Tuberculin Required for the D.I.T.

We are convinced that it is necessary to use a tuberculin of a least the same potency as that of the Frankfort standard to obtai a reasonable percentage of definitely positive reactions by the D.I.T In some cases we have found it possible to produce definite positiv reactions by diluting standard tuberculin once with concentrate glycerine veal broth, but this does not always hold, nor was a definit reaction ever produced by such a standard tuberculin diluted fou times. We have had occasion to use a tuberculin made by Captai Douglas, which we have shown to be at least twice, but possibly no four times, as strong as our standard tuberculin by the D.I.T. method Our colleagues (Okell and Parish) have prepared a tuberculin simila to that supplied by Douglas, and with it we have carried our man experiments in cattle. We have titrated this tuberculin agains "standard" in varying dilutions; the identity and strength of th dilutions were, of course, unknown to the reader of the reaction When used full strength it has always given a decidedly better reactio than standard, and a reaction classified as "doubtful" when standar tuberculin was used, could, on some occasions, be read as "positive when the Douglas type of "extra strong" tuberculin was injecte We believe that the stronger the tuberculin used, the smaller wi be the number of cows that will be read as "doubtful."

Considerable difficulty has been met with in the attempts to produce is “ extra strong tuberculin ” on a large scale, but present experience ggests that this difficulty will shortly be overcome.

The identifying of potency of tuberculins which have been purposely luted has been carried out. On all occasions we were able to say at one tuberculin four times as strong as the other was the better berculin, but we have not been able to tell on every occasion that berculin diluted once was less potent than the same tuberculin sed full strength. One hundred and nine tests in all were carried out.

Conclusions.—A strong tuberculin gives the most accurate results. uberculin cannot be titrated to a great degree of accuracy by the .I.T. method in cattle.

VI.—The Effect of One Intradermic Test on Subsequent Intradermic Tests.

The effect of an intradermic dose of tuberculin on the reaction tests at a later date has been investigated. In our earlier tests vo or more preliminary D.I.Ts were done on each cow, followed fortnight later by a single D.I.T. or, on some occasions by two D.I.Ts. rom such work we formed the impression that the preliminary testing used a “ lessening ” of the degree of reaction to subsequent tests reacting cattle.

A further study, in which 32 tests were done, was undertaken later. he following are our conclusions :—

(*a*) A single D.I.T. has no influence on a second single D.I.T. one week or a fortnight later.

(*b*) Three D.I.Ts. on the same cow at the same time may influence a single D.I.T. one week or a fortnight later.

(*c*) Five or more D.I.Ts. on the same cow at the same time may considerably influence a single D.I.T. one week or a fortnight later.

(*d*) In this limited number of experiments we could find no influence of first tests on several tests made a month or more later.

VII.—The Effect of the Subcutaneous Test on the D.I.T.

We have had the opportunity of studying the effect of a subcutaneous dose of tuberculin on subsequent D.I.Ts. The following is n example of one series of tests. Five heifers showing “ strongly ositive ” reactions to the D.I.T. were injected four to six months ater with diluted tuberculin (3 c.c.) subcutaneously. All gave classical emperature reactions. Two days later three were tested by the .I.T. method, two receiving three injections and one receiving a ingle injection. Four days after the subcutaneous test the remaining wo heifers were each given a single D.I.T. The results shown in

Table III indicate that the subcutaneous test has some influenc on a subsequent D.I.T. performed two to four days later. Furthe tests in which the D.I.T. was done more than one week after th subcutaneous test would indicate that at that interval there may b little effect of the one test on the other. These deductions are draw from tests done on 22 animals only.

It may be of interest to note that one cow which has been unde our observation for two and a half years, always gave a typical reactio to the subcutaneous and an entirely negative reaction to the intradermic test. Testing was carried out at least every six months durin the period of observation and one month or more elapsed on eac occasion between the application of the subcutaneous and intradermi tests. This cow was slaughtered and *post-mortem* examination reveale extensive tubercular lesions in the lungs, pleura, pericardium an intestinal wall. Acid fast organisms were demonstrated in thes situations and guinea pigs inoculated with the pathological materi have developed generalised tuberculosis.

CONCLUSIONS.—A dose of tuberculin subcutaneously may influen the reaction of a D.I.T. done two to four days later.

TABLE III.

Heifer.	Subcut. Test Reaction.	Interval.	D.I.T. Reactio
1	+	2 days	+
2	+	2 ,,	(*a*) ?
			(*b*) —
			(*c*) —
3	+	2 ,,	(*a*) +
			(*b*) ?
			(*c*) ?
4	+	4 ,,	—
5	+	4 ,,	—

All were positive to the D.I.T. four to six months before t subcutaneous test.

+ = Positive Reaction. — = Negative Reaction. ? = Indefinite Reacti

GENERAL DISCUSSION.

We feel that a practitioner commencing this work should not f to put all animals that he cannot certainly call "positive" "negative" into an "indefinite" or "doubtful" class, listed for ond test at a future date. With increasing experience he will put ver in the “ doubtful ” class, but even after considerable experience d even with a continuous supply of “ extra strong ” tuberculin, ere will probably always be a small number of cows that must be t into the “ doubtful ” class. And indeed one would expect this, r we know that if a group of guinea pigs be infected with living lture of tubercle they commence by being completely negative to tuberculin tests and end in, say, three or four weeks, by being sitive to all tests. There is an intermediate period during which subcutaneous or intradermic test gives a result so little differing om that on an uninfected animal that one cannot say with certainty ich is the infected animal, *i.e.*, the result is “ indefinite ” or doubtful.”

With regard to the question of “ doping ” or “ plugging ” berculous cattle with tuberculin so that they will not subsequently detectable by the ordinary tests, our experience early inclined us the belief that the D.I.T. may, under certain conditions, fail to tect a tuberculous animal that has been recently injected with berculin. It would probably take carefully controlled experiments a very large number of animals to make a decision on this point rtain and this we were unable to do. We therefore wrote to several thorities in the U.S.A. where the intradermic subcaudal single ection test has been used on a very large scale.

Dr. Kiernan, the Chief Veterinary Officer of the Tuberculosis adication Division, Bureau of Animal Industry, Washington, ndly gave us permission to quote his experience. He finds that berculous cattle, after either subcutaneous or intradermic test, may spond capriciously to subsequent tests. Some will respond repeatedly, hile others may fail to respond again for a long period. (Another nerican veterinary officer has informed us as the result of his perience that tuberculous cattle can be doped so that they will t respond to any kind of tuberculin test.)

With regard to “ indefinite reactors,” Dr. Kiernan states that the ndency of opinion is that all indefinite or suspicious reactors are st removed from a herd if one is attempting to build up a tuberculosis-ee herd, for the State records show that a large percentage, in some stances 50 per cent. of such indefinite reactors when tested again a somewhat later date are found to give positive reactions and to tuberculous.

The general conclusion would seem to be that although careful sting will often detect tuberculosis in a “ doped ” animal it cannot ways be depended on to do so.

GENERAL CONCLUSIONS.

(1) It would appear to be advisable for the veterinary surgeo using the double intradermic test to check his technique occasionall by doing duplicate injections and making duplicate readings. Mos bovines are readily classed as "positive" or "negative," a smal group of "doubtful" reactors needs careful consideration an retesting at a later date.

(2) The increase in swelling, etc., produced by the repeat injectio (R.I.) is specific.

(3) Tuberculins proved equal in potency by the official Frankfor method and by the intradermic test in guinea pigs are also equal b the D.I.T. method in cattle. To make a satisfactory comparison i cattle, a number of cows must be used. For titration of tuberculin the only satisfactory method at present available is the intradermi test in guinea pigs followed by the subcutaneous test in the sam animal.

(4) A strong tuberculin gives the most accurate results. Tuberculi cannot be titrated to a great degree of accuracy by the D.I.T. metho

(5) (*a*) A single "double intradermic test" (D.I.T.) has no influenc on a second single D.I.T. one week or a fortnight later.

(*b*) Three D.I.Ts. on the same cow at the same time may influenc a single D.I.T. one week or a fortnight later.

(*c*) Five or more D.I.Ts. on the same cow at the same time ma considerably influence a single D.I.T. one week or a fortnight late

(*d*) There may be no influence of first tests on tests made a mont or more later.

(6) A dose of tuberculin subcutaneously may influence the reactio of a D.I.T. done two to four days later.

(7) We have never been convinced that the milk yield of a her of milking cows has been materially lessened during the 24 or 48 hou following the intradermic test.

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